1.3 DEFICIENCIES IN THE PRIOR ART

Unfortunately, while many laboratories have attempted to make mutated crystal proteins, few have succeeded in making mutated crystal proteins with improved lepidopteran toxicity. In almost all of the examples of genetically-engineered *B. thuringiensis* toxins in the literature, the biological activity of the mutated crystal protein is no better than that of the wild-type protein, and in many cases, the activity is decreased or destroyed altogether (Almond and Dean, 1993; Aronson *et al.*, 1995; Chen *et al.*, 1993, Chen *et al.*, 1995; Ge *et al.*, 1991; Kwak *et al.*, 1995; Lu *et al.*, 1994; Rajamohan *et al.*, 1995; Rajamohan *et al.*, 1996; Smedley and Ellar, 1996; Smith and Ellar, 1994; Wolfersberger *et al.*, 1996; Wu and Aronson, 1992). For a crystal protein having approximately 650 amino acids in the sequence of its active toxin, and the possibility of 20 different amino acids at each of these sites, the likelihood of arbitrarily creating a successful new structure is remote, even if a general function to a stretch of 250-300 amino acids can be assigned. Indeed, the above prior art with respect to crystal protein gene mutagenesis has been concerned primarily with studying the structure and function of the crystal proteins, using mutagenesis to perturb some step in the mode of action, rather than with engineering improved toxins.

Several examples, however, do exist in the prior art where improvements to biological activity were achieved by preparing a recombinant crystal protein. Angsuthanasamnbat *et al.* (1993) demonstrated that a stretch of amino acids in the dipteran-toxic Cry4B delta-endotoxin is proteolytically sensitive and, by repairing this site, the dipteran toxicity of this protein was increased three-fold. In contrast, the elimination of a trypsin cleavage site on the lepidopteran-toxic Cry9C protein was reported to have no effect on insecticidal activity (Lambert *et al.*, 1996). In another example, Wu and Dean (1996) demonstrated that specific changes to amino acids at residues 481-486 (domain 2) in the coleopteran-toxic Cry3A protein increased the biological activity of this protein by 2.4-fold against one target insect, presumably by altering toxin binding. Finally, chimeric Cry1 proteins containing exchanges of domain 2 or domain 3 sequences and exhibiting improved toxicity have been reported, but there is

no evidence that toxicity has been improved for more than one lepidopteran insect pest or that insecticidal activity towards other lepidopteran pests has been retained (Caramori *et al.*, 1991; Ge *et al.*, 1991, de Maagd *et al.*, 1996). Based on the prior art, exchanges involving domain 2 or domain 3 would be expected to change insecticidal specificity.

The prior art also provides examples of Cry1A mutants containing mutations encoding amino acid substitutions within the predicted α helices of domain 1 (Wu and Aronson, 1992; Aronson *et al.*, 1995, Chen *et al.*, 1995). None of these mutations resulted in improved insecticidal activity and many resulted in a reduction in activity, particularly those encoding substitutions within the predicted helix 5 (Wu and Aronson, 1992). Extensive mutagenesis of loop regions within domain 2 have been shown to alter the insecticidal specificity of Cry1C but to not improve its toxicity towards any one insect pest (Smith and Ellar, 1994). Similarly, extensive mutagenesis of loop regions in domain 2 and of β-strand structures in domain 3 of the Cry1A proteins have failed to produce Cry1A mutants with improved toxicity (Aronson *et al.*, 1995; Chen *et al.*, 1993; Kwak *et al.*, 1995; Smedley and Ellar, 1996; Rajamohan *et al.*, 1995; Rajamohan *et al.*, 1996). These results demonstrate the difficulty in engineering improved insecticidal proteins and illustrate that successful engineering of *B. thuringiensis* toxins does not follow simple and predictable rules.

Collectively, the limited successes in the art to develop synthetic toxins with improved insecticidal activity have stifled progress in this area and confounded the search for improved endotoxins or crystal proteins. Rather than following simple and predictable rules, the successful engineering of an improved crystal protein may involve different strategies, depending on the crystal protein being improved and the insect pests being targeted. Thus, the process is highly empirical.

Accordingly, traditional recombinant DNA technology is clearly not routine experimentation for providing improved insecticidal crystal proteins. What are lacking in the prior art are rational methods for producing genetically-engineered *B. thuringiensis* Cry1 crystal proteins that have improved insecticidal activity and, in particular, improved toxicity towards a wide range of lepidopteran insect pests.

2.0 SUMMARY OF THE INVENTION

The present invention seeks to overcome these and other drawbacks inherent in the prior art by providing genetically-engineered modified *B. thuringiensis* Cryl δ-endotoxin genes, and in particular, *cryIC* genes, that encode modified crystal proteins having improved insecticidal activity against lepidopterans. Disclosed are novel methods for constructing synthetic Cryl proteins, synthetically-modified nucleic acid sequences encoding such proteins, and compositions arising therefrom. Also provided are synthetic *cryl** expression constructs and various methods of using the improved genes and vectors. In a preferred embodiment, the invention discloses and claims Cry1C* proteins and *cryIC** genes which encode the modified proteins.

An isolated nucleic acid segment that encodes a polypeptide having insecticidal activity against Lepidopterans is one aspect of the invention. Such a nucleic acid segment is isolatable from *Bacillus thuringiensis* NRRL B-21590, NRRL B-21591, NRRL B-21592, NRRL B-21638, NRRL B-21639, NRRL B-21640, NRRL B-21609, or NRRL B-21610, and preferably encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:59 or SEQ ID NO:61. Exemplary nucleic acid segments specifically hybridizes to, or comprise the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:58, or SEQ ID NO:60 or a complement thereof.

In certain embodiments, such a nucleic acid segment may be operably linked to a promoter that expresses the nucleic acid segment in a host cell. In those instances, the nucleic acid segment is typically comprised within a recombinant vector such as a plasmid, cosmid, phage, phagemid, viral, baculovirus, bacterial artificial chromosome, or yeast artificial chromsome. As such, the nucleic acid segment may be used in a recombinant expression method to prepare a recombinant polypeptide, to prepare an insect resistant transgenic plant, or to express the nucleic acid segment in a host cell.

A further aspect of the invention is a host cell which comprises one or more of the nucleic acid segment disclosed herein which encode a modified Cryl* protein. Preferred